Mesenchymal stem cells carrying oncolytic virotherapy for treating children with neuroblastoma: results of a compassionate use program.

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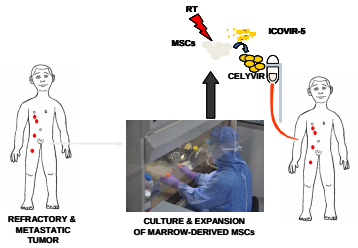
INTRODUCTION

We reported an initial clinical experience in the use of CELYVIR in 4 children with advanced neuroblastoma (NB)[1], a group of patients for whom new strategies are needed in order to improve outcome. CELYVIR is the acronym for autologous mesenchymal stem cells (MSCs) infected with ICOVIR-5, an oncolytic adenovirus[2] designed for systemic treatment of disseminated solid tumors. ICOVIR-5 contain several modifications that give it selective replication ability in cancer cells in which the Rb/E2F route is activated. Our strategy consists in systemic infusions of CELYVIR aiming at enhancing the targeted delivery of the oncolytic adenoviruses to the metastases based in the natural tumor tropism of the MSCs. Here, we now report the complete program of compassionate use of this new antitumor medicine, after treating 12 additional children. We have gathered information that confirms the safety of this procedure, enabling numerous infusions per child, amounting for very high doses of virus with very low toxicities. We have also found some clinical responses in our cohort. Patients who responded to treatment showed interesting differences in immunity, before and during treatment, compared to children without response. In addition, their MSCs showed differences in the expression of cell adhesion molecules and immune-related genes when comparing both groups of patients.

[1]. García-Castro J et al. Treatment of metastatic neuroblastoma with systemic oncolytic virotherapy delivered by autologous mesenchymal stem cells: an exploratory study. Cancer Gene Ther. 2010;17:476-83.
[2]. Cascallo M et al. Systemic toxicity-efficacy profile of ICOVIR-5, a potent and selective oncolytic adenovirus based on the pRB pathway. Mol Ther. 2007;15:1607-15.

RESULTS



CELYVIR strategy for metastatic neuroblastoma.
Bone marrow mesenchymal stem cells (MSCs) were obtained from the iliac crest of patients. MSCs production complied with the principles of Good Manufacturing Practice. MSCS received 30 Gy irradiation, were then infected with ICOVIR-5, washed and resuspended in saline supplemented with human albumin, and infused through a central line.

Changes in circulating lymphocytes during treatment with CELYVIR

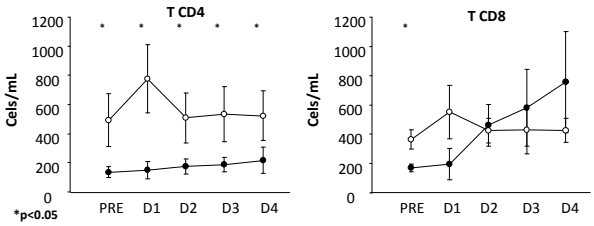


Figure 1. We followed the absolute numbers of circulating leukocytes before and after CELYVIR therapy and found changes in all patients. Numbers of leukocytes of the innate immunity such as neutrophils (NT), monocytes (MO), natural killer (NK) cells, and of the adaptive response like B-lymphocytes were not significantly different when comparing responder and non responder patients (not shown). Patients who responded had significantly higher counts of T lymphocytes pre-therapy (p=0.0157, Wilcoxon test). Absolute numbers of T lymphocytes remained higher, mainly in CD4 (not significantly for CD8), along therapy among the patients that showed a clinical response.

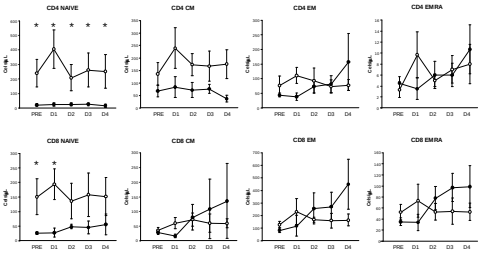


Figure 2. Naïve (CD45RA+CCR7+) CD4 and CD8 T lymphocyte numbers in children who responded were always above those of children with no response. Major changes in the numbers of these T cell subpopulations affected to the effector memory (CD45RA-CCR7-) and effector memory CD45RA (CD45RA+CCR7-) subsets of children who did not respond to the therapy, both in CD4 and CD8 T lymphocytes, and also in CD8 central memory (CD45RA-CCR7+). Compared to responder patients who presented an almost flat kinetic, non-responders showed a trend towards increasingly higher numbers of these subpopulations during therapy. These differences were not statistically significant. Finally, we also analyzed the subpopulation of circulating CD4+CD25+CD127low/negative containing T regulatory (Treg) lymphocytes, and did not find statistically significant differences between both groups of patients (not shown). * p<0.05.

Patient's and treatment

ID	GENDER	LINES TH	CELYVIR DOSES	CELLS (x10^6)	VIRUS	PCR PB	OUTCOME
UPN5	F	3	70	2,640	2.6 E+14	+	SD
UPN6a	M	3	6	226	4.5 E+12		CR
UPN6b	M	5	29	1,013	2.3 E+13	+	PD
UPN7	F	4	9	520	5.2 E+13	ND	PD
UPN10	M	4	6	160	1.6 E+13	ND	PD
UPN11	M	4	4	150	1.5 E+13	ND	PD
UPN12	F	4	10	340	3.4 E+13	ND	PD
UPN13	M	4	8	280	2.8 E+13	ND	PR
UPN16	F	3	7	150	1.5 E+13	+	PD
UPN18	F	4	14	300	3.0 E+13	+	PD
UPN19	M	4	9	220	2.2 E+13	+	PR
UPN20	F	3	6	290	2.9 E+13	+	PD

Table legend: ID, identification. TH, therapy. PCR PB, detection of adenoviral genome in peripheral blood by PCR. UPN, unique patient number. SD, stable disease. PR, partial response. CR, complete response. PD, progressive disease. ND, not done.

Table 1. CELYVIR therapy and follow-up.
Twelve patients diagnosed with refractory neuroblastoma were enrolled in a program of compassionate use. The local Research Ethics Board and the Spanish Medicine Agency (AEMPS) approved each patient's treatment in an individualized basis, and informed consent was obtained from each participant. Table I shows patients' and infusions' characteristics. Toxicities were studied recording clinical symptoms and signs of adverse effects, and by hematological and biochemical analysis done in blood samples previous to each infusion. Clinical responses were evaluated after the 6th dose with the level of serum enolase and with ^{123}I MIBG-scintigraphy, comparing the number of lesions pre- and post-therapy. Blood cell counts and serum biochemical parameters were in normal ranges following each infusion. Mild and auto limited viral-related toxicities were the only adverse effects detected. None of the 12 patients experienced grade 3+ toxicities. The most frequent toxicity observed was low-grade fever and flu-like symptoms in 10 patients.

Adhesion molecule profile of MSCs and clinical outcome

MOLECULE	RESPONDERS	NON RESPONDERS	P VALUE
CCR1	2.208	1.29	0.0157
CCR2	1.725	1.09	>0.1
CCR3	4.4	1.01	>0.1
CCR4	1.237	1.14	>0.1
CCR5	1.561	1.14	>0.1
CCR6	0.985	0.84	>0.1
CCR7	3.485	1.87	>0.1
CCR9	3.645	2.22	>0.1
CXCR1	1.027	0.67	0.0424
CXCR2	0.587	0.52	>0.1
CXCR3	0.964	0.88	>0.1
CXCR4	4.09	0.9	0.0882
CXCR5	1.432	1.14	>0.1
CXCR6	4.646	0.95	>0.1
α4 Integrin	2.235	3.01	>0.1
α5 Integrin	1.654	1.27	>0.1
β1 Integrin	18.457	23.35	>0.1
β2 Integrin	0.808	0.89	>0.1
β3 Integrin	0.178	0.16	>0.1
E-SELECTIN	0.986	1.03	>0.1
L-SELECTIN	0.608	0.97	>0.1
P-SELECTIN	0.202	0.17	>0.1
ICAM-1	4.0349	3.14	>0.1
ICAM-2	1.397	1.47	>0.1
ICAM-3	0.586	0.52	>0.1
VCAM-1	0.6368	0.51	>0.1
CD44	75.39	29.62	>0.1
CD105	1.027	0.81	>0.1
LFA-3	1.58	1.38	>0.1

Table 2. Comparisons of cell adhesion molecules studied by flow cytometry..
MSCs exhibit tropism for damaged tissues as well as the tumor microenvironment and many different receptors have been implicated in the homing of MSCs. We studied expression levels of several adhesion molecules on the surface of the CELYVIR product. The mean fluorescence intensity (MFI) was obtained for each adhesion molecule. Expression levels were normalised to those of their respective isotype control to allow comparisons. We found that irradiated and infected MSCs of patients that had a positive clinical response expressed significantly (*p<0.05) higher levels of CXCR1 and CCR1 than MSCs of patients with no response. In addition, the levels of CXCR4 tended to be higher, almost statistically significant (p<0.1), in the group of patients that responded after receiving CELYVIR.

Immune molecule profile of MSCs and clinical outcome

MOLECULE	RESPONDERS	NON RESPONDERS	P VALUE
CCL5	0.55	0.40	>0.1
CXCL10	0.45	0.31	>0.1
IDO1	0.14	0.67	0.0527
IFNγ	0.17	1.25	0.0167
IL-10	0.50	1.78	>0.1
IL-6	0.22	0.62	0.0894
IL-8	3.63	13.99	0.0874
TGFβ1	0.67	1.11	>0.1
TGFβ2	0.01	0.01	>0.1
TGFβ3	0.78	1.17	>0.1
TNFα	0.23	0.38	>0.1
VEGFα	1.24	2.04	0.0894

MOLECULE	RESPONDERS	NON RESPONDERS	P VALUE
CD80	1.192	1.17	>0.1
CD86	0.773	0.85	>0.1
B7H1	8.964	6.95	>0.1
B7H2	0.334	0.35	>0.1
B7H3	3.292	3.18	>0.1
B7H4	0.9114	0.83	>0.1
B7DC	1.694	1.87	>0.1
HLA-DR	1.603	0.81	0.0118

Table 3 and 4. We determined the levels of immune related genes expressed by the infused MSCs, using RTqPCR assays (upper table). Expression levels were normalised to those of MSCs form healthy donors. We found that IFNγ was expressed at significantly lower levels by the CELYVIR product of children with clinical responses, while IDO, IL6, IL8 and VEGFa levels showed a decrease in these patients which almost reached statistical significance (p<0.1). We also studied the expression levels of several immune-related molecules (B7H family, HLA) on the surface of MSCs of patients treated with CELYVIR by flow cytometry (lower table) and found that irradiated and infected MSCs of patients that had a positive clinical response expressed significantly higher levels of HLA-DR than MSCs of patients with no response (*p<0.05)

CONCLUSIONS

Our results confirm that the use of mesenchymal progenitor cells carrying an oncolytic adenovirus is a safe procedure in treating children with advanced NB, which can be administered in a multidose protocol with very high quantities of virus and an excellent tolerance. The presence of MSCs in the medicine product may have an impact in the response to the therapy beyond their role as cell carriers. Several characteristics of the MSCs, related to their migratory capacities and response to the viral infection, may help us in designing an optimum version of this new antitumor strategy

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